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Device for separating and discharging plasma

The invention relates to the field of plasma collection which plays an important role in analytical methods for determining a concentration of blood components. In many cases such blood tests cannot be carried out with whole blood since this contains corpuscular components (blood cells) which could result in an interference of the assay procedure. Hence in order to carry out many analytical methods it is necessary to firstly isolate plasma from whole blood which should be as free as possible from cellular material.

A conventional method for isolating plasma for blood tests is the centrifugation procedure in which cellular components of the blood are separated on the basis of centrifugal forces. This method is laborious and is especially unsuitable when only small amounts of plasma are required for an analysis. However, particularly modern miniaturized tests use quantities of plasma that are only in the range of a few microlitres. This applies especially to so-called carrier-bound tests in which an analytical system that is as small and compatible as possible is present for example in the form of a test strip. In this case all reagents and other agents required to carry out the test are integrated into the test strip. In order to determine an analyte the sample liquid is contacted with such an analytical element. The reagent contained in the test element reacts within a short period with the analyte to be determined such that a physically detectable change occurs on the analytical element. Such a change can for example be a colour change or a change in a measurable electrical variable. The change is measured and calculated with the aid of an evaluation instrument in order to output an analytical result.

An example of an analytical system which determines an analyte from plasma by means of such a test element is the HDL test (high density lipoproteins). The determination of the HDL concentration in blood is, among other things, important

for the risk assessment of coronary heart disease and is thus in recent times used to diagnose one of the most common modern diseases.

The severity of a coronary heart disease can be assessed on the basis of several known parameters such as total cholesterol, in the blood, plasma or serum. Since the concentration of total cholesterol is of only limited use for individual risk assessment, the low density lipoproteins (LDL) and the high density lipoproteins (HDL) are quantified separately from one another in modern analytical methods. When assessing such analytical methods it must be taken into account that there is a positive correlation between LDL cholesterol and a coronary heart disease but a negative correlation between HDL cholesterol and the disease. Clinical studies have proven that, as a first approximation, the determination of HDL and total cholesterol is sufficient for a risk assessment. This is the preferred method in current diagnostic practice.

HDL cholesterol is for example determined by means of an analytical element such as those known in the prior art (e.g. HDL test elements from Roche Diagnostics GmbH). Since the HDL cholesterol is determined separately, the other lipoprotein classes that are present have to be separated from the remaining blood components to allow the determination of HDL cholesterol in plasma. Such a test for example requires a plasma volume of about 40 µl in order that the concentration can be determined independently of the applied plasma volume. Only pure plasma in which there are essentially no blood components can be used to determine HDL cholesterol. A complexing agent which is also integrated into the test element is additionally used to determine the HDL concentration. Plasma is then applied to the zone of the test element in which the complexing agent is present. The complexing agent EDTA is for example used in the prior art to analyse HDL cholesterol.

However, an analyte in pure plasma can also be determined by means of a test element that requires no complexing agent to determine an analyte. Such test elements are for example used to determine enzymes and are described in the prior art in the document DE 3130749 among others. Test elements which determine an

analyte in pure plasma but do not contain a complexing agent are often designed such that the test element itself separates plasma. For this purpose such test elements contain a separation layer in addition to a reagent layer. In order to measure an analyte whole blood is firstly applied to the separation layer. Blood components are separated from the plasma within the separation layer and the plasma is passed on to the reagent layer. In this manner an analyte can be determined in pure plasma although blood has been applied to the test element. However, such a plasma separation layer integrated into the test element cannot be used when using complexing agents. If a complexing agent is used to determine an analyte, it turns out that the complexing agent in the test element prevents the separation of plasma from blood. Hence the test element can no longer separate plasma if the test element contains a complexing agent.

Since, on the one hand, a complexing agent is needed to determine HDL cholesterol by one of the test elements described above and, on the other hand, it is necessary to separate plasma from blood, it follows that the plasma has to be already separated from blood on a µl scale before applying blood to the test element.

In this connection the determination of HDL cholesterol is only one important example of an analyte determination which requires small amounts of pure plasma for analysis. Other fields of application for the use of released plasma are in the field of clinical analysis. Since test strips are currently preferably used in diagnostic practice as analytical systems, there is an increasing need for simple methods for obtaining small amounts of plasma in order to achieve an overall simplification and more rapid analytical procedure.

In this respect several methods have been described in the prior art which are intended to simplify the isolation of small amounts of plasma. The aim is to obtain plasma from likewise small volumes of blood to spare the patients a laborious blood withdrawal which would for example be required for a centrifugation method.

Filtration methods in which different filter media and in particular membrane and glass filters are used have been discussed for many years and in some cases have been used successfully. Earlier examples of filtration technology are described in the US patent documents US 3,791,933 and US 4,477,575. A recent example comprising a complicated combination of membrane and glass filters is described in the patent US 5,922,210. Small amounts of plasma are obtained by microfiltration with the aid of a microcomponent. The blood cells are separated by a so-called barrier channel which is too small to allow blood cells to flow through the barrier channel. However, manufacture of a device with such a channel requires special manufacturing processes which are complicated. The said plasma collection methods have the additional disadvantage that there is a high risk of the fine pores being clogged up by mechanical plugging or by accumulation of cellular material on the walls of the pores. This would reduce the filter capacity. However, an enlargement of the filter capacity would require more space for the filter medium. This would in turn have an unfavourable effect in relation to the applied sample volume and volume of plasma obtained.

A filtering process for collecting plasma is described in the document US 4,477,575 which comprises a glass fibre layer which improves the relation between sample volume and the volume of plasma obtained. In this case the volume of plasma to be separated is preferably less than 30 % of the suction volume of the glass fibre layer. The filtering process takes place after blood has been applied to the glass fibre layer and is driven solely by gravity and hence the plasma isolation is correspondingly time consuming.

In order to accelerate plasma collection other methods for collecting plasma have been described in the prior art. In the patent application EP 747105 a glass fibre onto which blood is applied is firstly stored in a vessel. Pressure is exerted by a plunger on the glass fibre and the blood contained therein to accelerate the filtration process. The blood is thus pressed through the glass fibre resulting in a separation of plasma from other blood components. The plasma is discharged via an outlet. However, a disadvantage of the described device is that large amounts of blood sample are

required due to the filtering process. In addition pressing out the glass fibres results in a destruction of the corpuscular blood components and hence it is not possible to obtain pure plasma.

A vessel for plasma collection which is described in the patent application EP 0 785 012 is based on a similar principle. In this case pressure is also exerted on a filter material to which blood has previously been applied to allow plasma separation to occur. As already described above the pressing out process destroys blood cells and hence pure plasma is not obtained. Once plasma has been contaminated by the destruction of blood cells, it is unsuitable for use in numerous analytical tests.

The object of the invention is to provide a device and a method which allow plasma that is as pure as possible to be obtained on a µl scale from whole blood. It should avoid the described disadvantages of the prior art.

The invention comprises a device for separating and discharging plasma. The device comprises a separation element containing a first zone on which blood is applied. Corpuscular blood components are essentially completely retained in the first zone of the separation element whereas plasma is passed into a second zone of the separation element advantageously by means of capillary forces. The separation element is arranged in the device in such a manner that the first zone of the separation element is accessible to the user for blood application. The device also has a discharge unit which, after plasma separation, acts on the second zone of the separation element without having an effect on the first zone of the separation element that would for example lead to blood haemolysis. If the discharge units acts exclusively on the second zone of the separation element, the separated plasma is released from the second zone and discharged through an outlet of the device.

The invention also encompasses a system for detecting analytes in blood. In addition to the device according to the invention, the system, as already described, also comprises a test element which enables detection of an analyte in plasma when the plasma separated by the device is applied.

The device according to the invention ensures an effective collection of plasma on a µl scale even from small sample volumes. For example plasma volumes of 30 µl or more can be obtained from 100 µl blood. Hence the device according to the invention is particularly suitable for the field of modern analyses since it already enables a rapid plasma separation and discharge of the plasma advantageously onto a test strip even when only small volumes of sample are drawn. If the applied blood volume is preferably 30 to 150 µl, the device according to the invention can be used to obtain sufficient amounts of plasma that are specified for commercial test elements in order that an analyte concentration can be determined independently of the applied sample volume. Consequently the device according to the invention enables sufficiently large amounts of plasma to be obtained despite small blood volumes in order to meet the requirements of commercial analytical methods especially with test elements.

Furthermore the device according to the invention allows a particularly simple and cost-effective manufacture of the system since for example small microstructures (microchannels) do not have to be integrated into the device in the manufacturing process. The plasma is separated by means of a separation element that is preferably designed for single use. Thus a blockage of the microporous structures due to multiple use and contamination can be avoided.

Other important advantages of the invention result from the release of the plasma and the separation of the plasma from blood which according to the invention are carried out as two separate successive processes. Thus it is possible to accelerate plasma collection without having to apply pressure on the sample during the separation process which is common in the prior art. According to the invention the plasma is released essentially only by means of an action on the second zone of the separation element whereby this process can be accelerated in any desired manner e.g. by overpressure, negative pressure or elution processes etc. The plasma separation step is essentially independent of this process and occurs independently of the release process and can for example also be accelerated by capillary forces which act inside the first zone of the separation element. However, care should be taken that

acceleration of plasma separation should only occur to the extent that a reliable separation of plasma from other blood components is still ensured. In particular processes which would cause haemolysis during plasma separation should be avoided e.g. those which require shear forces. Consequently the invention enables an accelerated plasma separation process without having to accept contamination of the plasma with other blood components.

In the field of modern analysis the invention is particularly suitable for applying pure plasma to test elements that, as described, contain a complexing agent and therefore cannot themselves separate plasma within the test element due to the complexing agent.

However, an application for test elements which do not contain a complexing agent is also conceivable. Although the plasma can also be separated commercially by means of a separation layer in the test element and consequently the user does not have to rely on a separate plasma separation in order to use these test elements, the system according to the invention allows a simplified test element construction in this case. Hence the test elements only need to have a reagent layer and no longer need to be provided with a separation layer or separation fleece. This reduces, among others, the number of production steps which lowers the costs of the test elements.

Using the test elements with a separation layer or separation fleece described in the prior art as a basis, a preferred embodiment of a separation element according to the invention is constructed as a first approximation in a similar manner. Reference is for example made to the document DE 3130749 for a more detailed description of commercial test elements with a separation layer. The document describes a test element in which plasma is firstly separated from blood so that only plasma is transported to a reagent layer. The reagent is then used to determine an analyte in plasma. For this purpose the test element has a flat separation layer located on the base strip on which the blood sample is applied at one end. The separation layer is composed of glass fibre material which retains the blood cells near to the site of application. In contrast blood plasma spreads in the layer in such a manner that a

"plasma lake" is available in the area of the separation layer that is distant from the separation layer. A reagent layer is usually located above or below the plasma lake which can be subsequently used to determine an analyte in the plasma. Appropriate evaluation devices that are known in the prior art are used to evaluate such a test carrier.

However, a disadvantage of these analytical systems is that the so-called "plasma lake" collected in this manner can only be used on the described system. Hence an analysis of analytes present in the plasma is only possible within the scope of the test carrier system since it is not possible to release plasma from the test carrier.

If a device according to the invention contains a separation element which is designed like such a simplified test element, such a separation element for example contains a separation layer in its first zone which is also referred to as a separation fleece in the following and it contains a transport fleece in a second zone in which the plasma collects in an area that is distant from the separation layer. Consequently a preferred embodiment of the separation element has a test carrier-like, strip-like structure without a reagent layer being present. The separation element preferably contains a filter in its separation fleece which for example is composed of glass fibre material to ensure an essentially complete separation of plasma from blood. Other commercial fleeces are for example described in the document EP 0 045 476 or are commercially available under the name Whatman fleece. In this case the filtering process according to the invention is essentially not assisted by pressure to avoid destruction of blood cells in the first zone of the separation element. If for example negative pressure is applied to the first zone of the separation element to assist the filtration process, then care should be taken that pressure is only exerted to an extent that does not cause haemolysis.

Plasma is then subsequently released by an action on the second zone of the separation element without influencing the first zone of the separation element. Thus according to the invention a contamination of the plasma by the process step of plasma release is prevented.

Pressure is preferably applied to the second zone of the separation element in order to release plasma such that plasma is pressed out of the second zone of the separation element. If plasma is pressed out of the separation element care should be taken that pressure is only exerted on the second zone of the separation element in which there are no more blood components to avoid haemolysis. In principle a variety of methods are conceivable for releasing the plasma whereby the process step of plasma release takes place according to the invention independently of the plasma separation so that the plasma separation step does not impose any constraints on the plasma release. Another example of plasma release is to elute the separated plasma. The released plasma is subsequently discharged in a dosed manner through an outlet of the device.

It has also proven to be advantageous for the release of the plasma when forces for releasing the plasma act on the second zone of the separation element essentially perpendicularly to the plane in which the separation element is located. This ensures that there is no effect on the first zone of the separation element which could otherwise contaminate the released plasma with the other blood components.

In this connection an embodiment using a plunger is conceivable in which the plunger is arranged within the device above or below the plane in which the second zone of the separation element is located. Hence pressing the plunger against the separation element only exerts pressure on the second zone of the separation element and thus plasma is released.

It is also conceivable that the second zone of the separation element is firstly separated from the first zone of the separation element in order to ensure the release of pure plasma so that for example the separation element zones can be spatially separated. In this case it is advantageous to take care that the second zone is only separated from the first zone at the site of the separation element in which essentially only plasma is present so that no corpuscular components can contaminate the plasma in the second zone of the separation element. The spatial separation now

allows the plasma to be released in a variety of manners without the risk of affecting the first zone.

The second zone of the separation element can for example be separated from the first zone by a holder which is connected to the second zone of the separation element. If the user exerts a force (pulling, pressing, twisting etc.) on this holder this force is directly or indirectly transferred to the second zone of the separation element and thus results in a detachment of the second zone. For example it is possible that a rotation of the holder by preferably 90° detaches the second zone from the first zone which is fixed in a permanent position within the device.

In another advantageous embodiment the separation element is detached and the plasma is subsequently released from the second zone in two successive steps which are carried out by actuating the same trigger unit on the device. Such a trigger unit is then linked to the holder of the device in such a manner that when the trigger unit is actuated first it initially results in a severing of the element and another actuation of the trigger unit results in plasma release. Trigger units are advantageous in the form of a release button which in a first step for example causes a rotation of a holder which is connected to the second zone of the separation element resulting in a rotation of this separation element. During the rotation of the second zone attached to the holder the first part of the separation element remains in a permanent position in the device. The forces exerted by the rotation result in a severing of the separation element. In this case it is for example conceivable that a cutting element is positioned in the device in such a manner that the second zone of the separation element is pressed against the cutting element during the rotation. This facilitates a severing of the separation element and can be carried out precisely. If a cutting element is omitted the separation element can also be severed by tearing the first zone from the second zone. Subsequently the plasma is released by the discharge unit.

If the separation element is designed as a single-use article in an advantageous embodiment of the device, an irreversibly severing of the element is not disadvantageous; it is also possible to offer the holder as a single-use article that is

permanently connected to the separation element. This would greatly simplify the handling of the devices for the user when reinserting a new separation element since especially older persons have difficulties in handling small instrument components. Moreover the holder and separation element can be stored in a dispenser which dispenses individual units of the single-use article.

Another subject matter of the invention is a method for separating and discharging plasma. The method comprises applying blood to a first zone of a separation element which contains a first and a second zone. The plasma is separated from other blood components by the separation element during which the plasma is passed into the second zone of the separation element and the remaining blood components are essentially retained in the first zone of the separation element. Subsequently the second zone of the separation element is processed in such a manner that plasma is released from the second zone of the separation element. In this connection it is important that no processing of the first zone of the separation element occurs which would cause plasma to be contaminated with previously separated blood components by for example haemolysis. The released plasma is discharged through an outlet of the device.

Advantageous embodiments of the method are derived as described. The method for plasma separation and release is preferably carried out by means of the described device.

The device according to the invention and the method according to the invention consequently allow a simple and rapid separation of plasma from blood on a microlitre scale. The device is convenient to handle and can be manufactured economically. Advantageous embodiments are shown in the figures and described in the following.

Figure 1: Structure of a separation element

Figure 2: Device for separating plasma

Figure 3: Device for separating plasma with a rotatably pivoted holder

Figure 1 shows an example of the construction of a separation element (1). The separation element (1) contains a transport fleece (2) which for example consists of glass fibres. A separation fleece (3) which is composed of a filter medium is mounted on the transport fleece (2). The main difference between the transport and the separation fleece is their different densities. In the prior art a density of 77 g/cm^2 is for example given for a separation fleece and 53 g/cm^2 for a transport fleece (Whatman fleece). The smaller thickness of the transport fleece allows a rapid transport of the sample along the fleece whereas the larger thickness of the separation fleece ensures a reliable separation of plasma from blood. When a blood drop (5) is applied, the blood enters the separation fleece (3). The filter medium in the separation fleece separates the blood components from the plasma and retains them in the separation fleece (3). The plasma is passed on by means of capillary forces which act within the transport fleece. In this case it has been often observed that small concentrations of blood components from the separation fleece (3) can enter a small area of the transport fleece (2) due to capillary forces. This area is referred to as a transition zone (6) and does not contain pure plasma. In an advantageous embodiment of the inventive device the discharge unit therefore does not act on the transition zone of the transport fleece during release of the plasma in order to avoid contamination of the remaining plasma with impurities that are contained therein. As shown in figure 2 the transition zone is avoided by for example detaching the second zone of the test element from the first zone on the other side of this transition zone to ensure that pure plasma is obtained.

Figures 2a) to c) are examples of a method for plasma separation using a device (10) according to the invention. The device contains a hollow body (14) which is provided with an outlet (13). The separation element (1) is arranged within the hollow body (14) in such a manner that the separation fleece (3) protrudes from the hollow body (14) and is easily accessible for the user. The transport fleece (2) is located within the

hollow body (14). The device also contains a plunger (12) which is movably mounted within the device (14). The radius of the plunger (12) is essentially identical to the inner radius of the hollow body (14) such that the plunger (12) can be moved by means of a button (11) within the device. Figure 2b) shows application of blood (5) on a separation fleece (3) of a separation element (1). If the blood enters the separation fleece, the plasma is passed along the separation fleece whereas the remaining blood components are retained in the separation fleece. A complete plasma separation occurs after about 2 to 10 sec. The separated plasma is now transported into the transport fleece (2). Actuation of the button (11) firstly presses the plunger (12) against the transport fleece (2) such that this area of the separation element is swept along by the plunger within the hollow body (14). Since the separation fleece (3) is permanently positioned in the hollow body, this results in a detachment of the transport fleece from the separation fleece whereby it is severed on the other side of the transition zone (6) shown in figure 1. Further actuation of the button (11) presses the separated transport fleece (2) against the wall (16) of the housing (14). In this process the plunger (12) presses the plasma out of the transport fleece (2) and releases it. Subsequently plasma (7) is discharged from the outlet (13) of the device. The plasma can then for example be applied to a test element (17) to determine the HDL concentration.

Figure 3 shows various views of another embodiment of the device (a – c). Compared to the embodiment shown in figure 2 the device additionally contains a rotatably pivoted holder (21) in which a separation element (1) is positioned within a channel (23). The separation element (1) is positioned in the holder in such a manner that the separation fleece (3) protrudes outside the holder and device in such a manner that it is readily accessible for blood application by the user as illustrated by the side-views. The device is also provided with a plunger (12) which is connected with the button (11). Furthermore the button (11) can operate a rotating element (22). Firstly blood is applied to the separation fleece (3) of the separation element (1) as shown in side-view in figure 3a). After the plasma has been separated from blood, the rotating element (22) is operated by a first pressing of the button (11). The holder (21) is rotated by about 90° by a downwards movement of the rotating element (22). This

detaches the separation fleece (3) from the transport fleece (2) during which the transition zone (6) of the transport fleece (2) remains attached to the separation fleece (3). Further actuation of the button (11) results in the plunger (12) which is firstly within a channel (23a) being transferred into the channel region (23b). This presses together the transport fleece against a sieve (24) located in the outlet (13). The sieve (24) preferably has a small thickness of 20 to 300 µm in order to avoid an excessive dead volume. The plasma is discharged through the outlet (13).